Bacteria isolated from the deep-sea hydrothermal field of Kolumbo submarine volcano: a potential source of new bioactive compounds

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INTRODUCTION

The escalating problem of drug resistance, especially in infectious diseases, requires a constant influx of new compounds to inspire the next generation of therapeutic agents. Over the past 25 years, more than 50% of all new chemical entities approved as drugs were based on natural products. The marine environment has emerged as a key source of natural product drug leads. Screening unique organisms from rare or extreme ecosystems is a proven approach to discover novel compounds with important biological effects. Deep-sea hydrothermal vents are among the most dynamic and extreme environments on Earth. Because unique microbial organisms inhabit the regions near these vents, there is a strong likelihood that new bioactive small-molecule natural products will be discovered, given that it is well established that chemical diversity is directly proportional to biological diversity. A remotely operated vehicle (ROV) exploration of Kolumbo submarine volcano (500 m depth) in the Aegean Sea revealed a very active high-temperature hydrothermal vent field, emitting colourless gas plumes with temperatures recorded as high as 220°C. (Fig. 1) The goal of this study was to screen cultured vent mesophilic bacteria (Fig. 2) for antimicrobial activity.

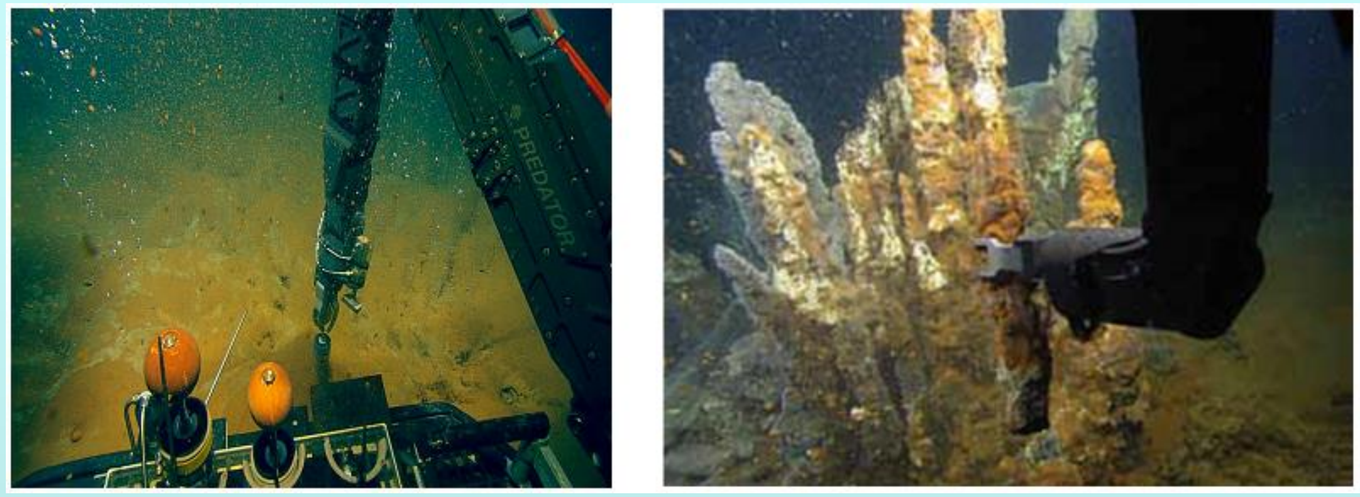

Fig.1 Sediment and chimney sampling

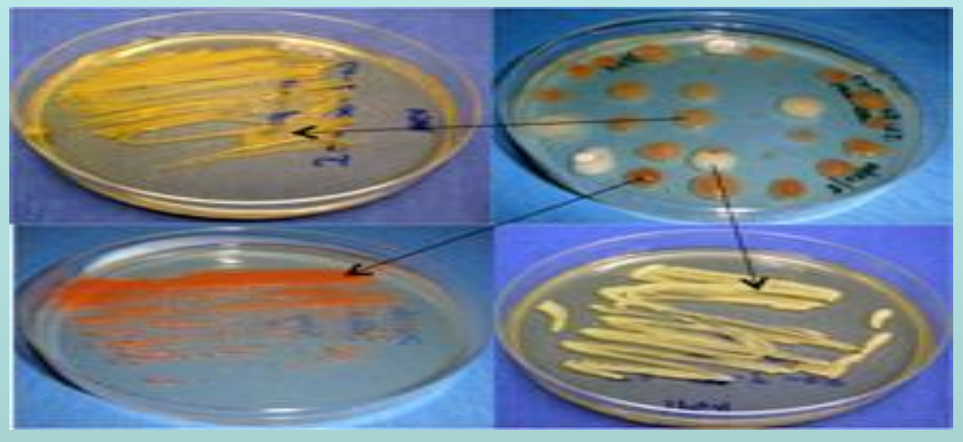

Fig. 2. Isolated cultured vent bacteria

MATERIALS and METHODS

➢During E/V Nautilus 2010 expedition in the Aegean Sea, sediments and chimney samples were collected by push core on Hercules Dive 1705 on July 31, 2010 (Core No. 12) (RV Nautilus/Hercules, July 28–August 13, 2010). The *in situ* temperature gradient was determined using the external Hercules temperature probe. The push core was returned to the surface and stored at 4 ºC in sterilised filtered sea water with 0·05% (w/v) Na_2S.

➢Samples were plated on selected media (Marine Agar and Tryptone Glucose Agar) and incubated aerobically for 1 week in room temperature.

➢832 bacteria were isolated and differentiated through BOX-PCR analysis at the strain level using primer BOX A1R 5'-CTACGGCAAGGCGACGCTGACC-3 collected from the hydrothermally active field of Kolumbo' (Rademaker & de Bruijn, 1997) and similarity coefficients were computed.

➢Bacterial isolates were tested for antimicrobial activity by diffusion method (Katsifas E.A., 1999), growing on agar plates and coated with 0,7% soft agar containing 18 different type strains (pathogenic and non pathogenic) of Gram positive and negative bacteria, fungi and yeasts obtained from the German Collection of Microorganisms and Cell Cultures DSMZ. The most bioactive isolates that showed antimicrobial activity against at least 6 type strains were then selected for sequencing analysis.

➢The 16s rRNA genes were amplified with the specific PCR primers pA 5´ - AGAGTTTGATCCTGGCTCAG-3´, (Edwards *et al.*, 1989) and R1492 5´ - TACGGYTACCTTGTTACGACT-3´ (Heuer *et al.*, 1997). DNA sequencing of 16S rRNA genes was carried out by using the primer R1492. The sequences were compared to 16S rRNA reference sequences of cultured bacterial strains deposited in the GeneBank database.

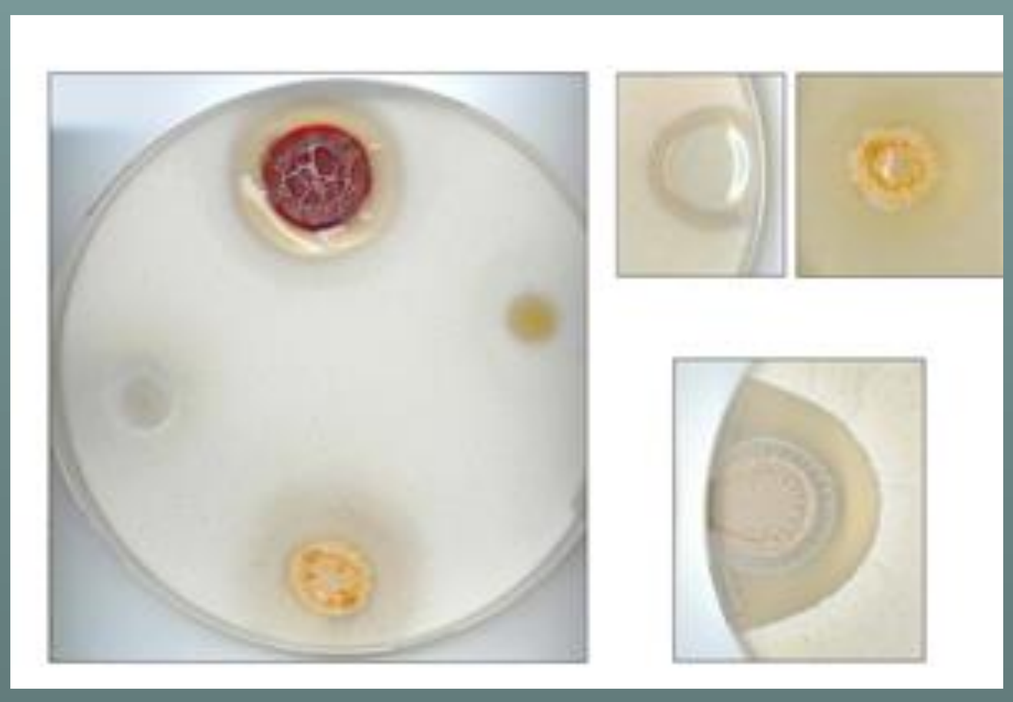

Fig. 4. Antifungal activity against the test organism *Aspergillus niger*

RESULTS and DISCUSSION

➢230 different BOX-PCR genomic fingerprints corresponded to the initial 832 isolates and a dendrogram was generated depicting the cluster analysis of these strains (Fig. 3).

➢187 of them showed remarkably reproducible antimicrobial activity (Fig. 4), 41 of which were found to be active against at least 6 type strains (Fig. 5). There has been already evidence for more prevalent chemical defenses in biofilm vs. planktonic populations of bacteria as well as for the role of bacterial bioactive compounds in the killing of eukaryotic predators (Matz *et al.*, 2008). The reason for different sensitivity between Gram-positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components which makes the cell wall impermeable to lipophilic solutes while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria are more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Trust and Sparrow, 1974).

➢The 16S rRNA genes of 41 representative bacteria were sequenced and the closest type strains of isolates were retrieved from NCBI. These bacteria belong to the phyla Firmicutes, genus *Bacillus* and Proteobacteria, genera *Halomonas*, *Pseudomonas* and *Loktanella*. It is interesting to note that these strains showed prominent activity against fungal and bacterial pathogens. Results of this study indicate that the potential of these microorganisms to produce antimicrobial activity is great and should be better explored. Future studies will be based on the identification of the active metabolites which have been responsible for the inhibitory effects.

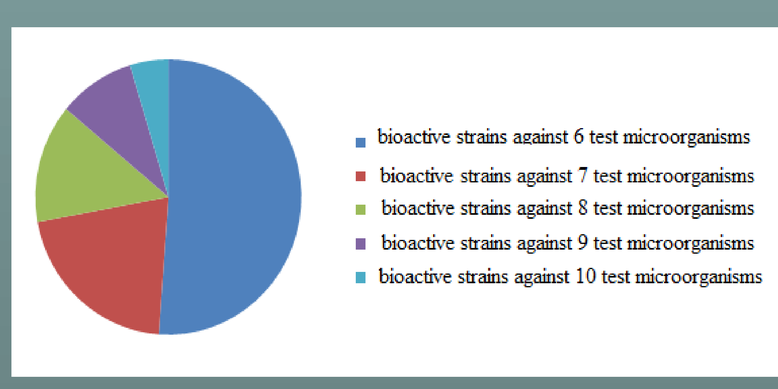

Fig. 5. Isolated bioactive strains against 6, 7, 8, 9 and 10 test organisms

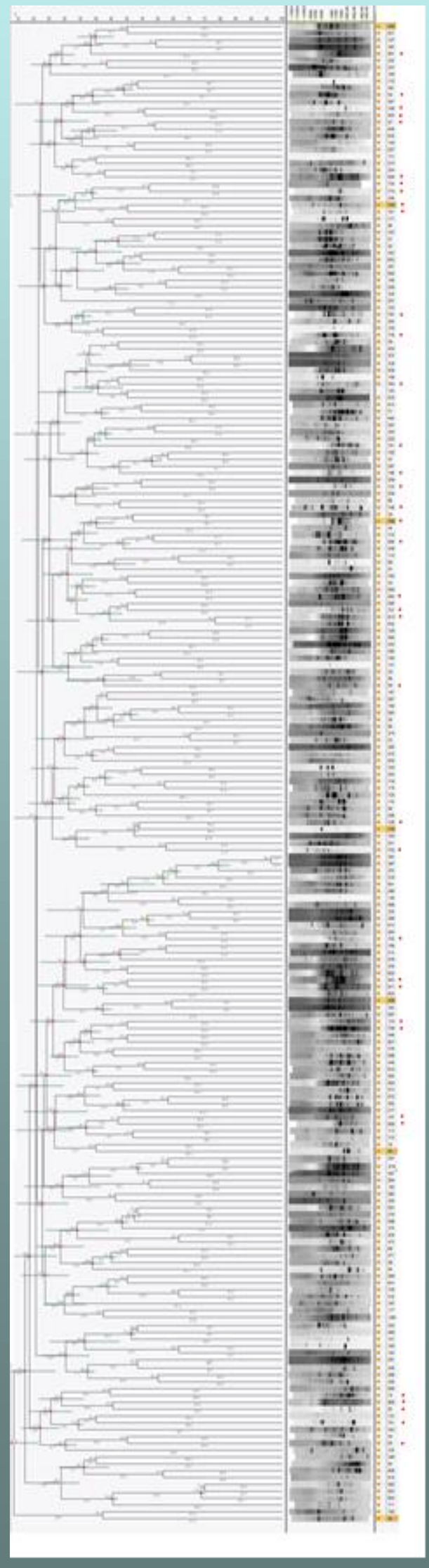

Fig.3 Dendrogram depicting the 230 different BOX-PCR genomic fingerprints

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